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Study of Aero mycological Biodiversity in Outdoor and Indoor Sources

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Abstract:

The fungal spores and hyphal fragments are commonly recorded in the air and are important for survival and subsequent continuation of generations. Such fungal spores are one of the major important components responsible for allergic disorders as spores are inhaled and deposited on sensitive mucosa. Many such airborne microorganisms are responsible for biodegradation of storage materials, equipment, library materials, painting etc. In the present studies, biodiversity of fungi appearing in indoor and outdoor premises of college campus was investigated. The study was carried out during the month of October, November and December which are representative of winter season. This particular season involves maximum students' activities. Wide fungal diversity was observed in both indoor and outdoor area during winter season. The present study gives comprehensive overview of presence and distribution of various fungal spores in both Indoor and Outdoor areas during winter season. This study would be important for effective management of fungal spore related problems affecting human life.

Key words:
Aeromycology, Antimicrobial, Biodiversity.

Introduction:

Population explosion urbanisation and industrialization have brought many changes in the environment. Like chemical particulate pollutants, the airborne bio components such as pollen grains, fungal spores are responsible for various types of allergic disorders among man (Jain *et. al.,* 1998) Exposure of bioaerosol, containing airborne microorganisms and their by products, can result in respiratory disorders and other adverse health effects such as infections, hypersensitivity, Pneumonities and toxic reactions (Gorny*et al.,* 2002, Fracchia *et. al.,* 2006). As it is known that a fungal spore forms an important constituent of bioaerosol, because of their volume in the atmosphere and small size, fungal spores play an important role in respiratory allergy and cause a wide range of symptoms, including allergic rhinitis, asthma, chronic bronchitis etc. (Tilak S. T. 1991, Vijay *et al.,* 1991, Hasnain *et al.,* 1994).

Due to increasing awareness of the relationship of airborne fungi to allergy scientist began to study the spectrum and incidence of airborne fungi word wide. Extensive survey conducted in India & abroad on respiratory allergies indicates that patterns in the incidence of 'airborne allergens' differ considerably from place to place & season to season. Many airborne

microorganisms are responsible for biodegradation of storage materials, equipment, library materials, painting etc. More than 80 genera of fungi have been associated with the most commonly identified belonging to three distinctive fungal groups, *Ascomycetes, Basidiomycetes and Deuteromycetes.* (Tilak S. T. 2010) The main types of allergic spores are *Aspergillus, Cladosporium,* & *Penicillium.* The clinical investigation have provided the significance and utility of treatment is preventing the effect of aeroallergens to sensitive individual. (Hung *et. al.,* 2011,Chakraborti *et al.,* 2012) Therefore it is a need of trained aeromycologist and clinicians.

Material and methods:

Media: Dehydrated Potato dextrose agar and Potato dextrose broth medium of Hi media were used for isolation of fungal species. All media were sterilized in autoclave at 15lb pressure for 20 minutes.

Reagents: Lacto phenol cotton blue stain solution of Hi media was used for staining of fungi.

Isolation and identification of Microorganisms:

1) Isolation of fungus species:

1. Three sets of Potato Dextrose Agar (PDA) plates were exposed to air for 10 minutes at five different places, Library, Office, Near Parking, ground and garden in college premises. The process was repeated in October, November and December.
2. These exposed plates were incubated at 27 - 30° C for 3 – 4 days.
3. Different isolated colonies of fungus were studied further.

2) Identification of Fungus species:

1. Individual colony showing different cultural characteristics was picked up with sterile loop and streaked on another Potato dextrose agar plates for obtaining pure culture. These plates were incubated at 27 - 30° C for 2 – 3 days.
2. Cultural characteristics of all fungal isolates were studies after incubation and loopful of culture was picked up from plates and streaked on Potato dextrose agar slant and used for further study after incubation at 27 - 30° C for 2 – 3 days.

Microscopic Identification of fungal morphology:

1. Place a drop of lacto phenol cotton blue on a clean slide.
2. Transfer a small tuff of the fungus preferably with spore and spore bearing structure into the drop using a flamed cooled needle.
3. Gently tease the material using the mounting needle.
4. Place a cover slip over the preparation taking care that air bubbles do not trap in.
5. Observed under low and high power objective.

6. Identification of fungal isolates was done microscopically using Lacto phenol cotton blue stain and their cultural characteristics.

Result and discussion:

Result showing numbers of fungal isolates differentiated on the basis of colour of colonies on PDA plates obtained in different areas are given in Table: 1.

Five different sampling sites in college premises were selected i.e. Library, Office, and area near Parking, Open ground and Garden. The study was conducted during the winter season, i.e.in the month of October, November and December. From the above table, it was observed that total 138 different coloured colonies were appeared in month of October with maximum in Office and least in Library. Similarly in the month of November, Library showed maximum number of fungi growing on plates and minimum in area near parking with total 127 numbers of isolates. In December there was not significant variation in no. of fungal isolates appearing on PDA plates in different areas with total 94 isolates. Wide fungal diversity was observed in both indoor and outdoor area during winter season.

The isolates were identified on the basisof cultural characteristics on PDA and microscopic examination. Results of cultural characteristics and microscopic examination are given in Table 2. Distribution of different fungal species at different locations and in different months are given in Table – 3.

In Library area:

In the month of October, the fungal isolates obtained in library were identified as *Penicillium* sp., *Pythium* sp. and *Aureobasidium* sp. In November the dominating fungal species were *Pythium* sp., *Trichoderma* sp., and *Dreshlera* sp., *Penicillium* sp., *Aureobasidium* sp., *Trichoderma* sp. And *Aspergillus* sp. were dominantly present in December.

In Office:

In the month of October, the fungal isolates obtained in office were identified as *Pythium* sp., *Fusarium* sp., *Aspergillus* sp., *Aureobasidium* sp., and *Curvularia* sp. In November the dominating fungal species were *Fusarium* species, *Trichoderma* species and *Penicillium* sp., In December, *Fusarium* sp, *Curvularia*, *and Aureobasidium* sp. were conformed.

Near Parking:

In the month of October, the fungal isolates obtained near Parking were identified as *Pythium* sp., *Aspergillus* sp., *Trichoderma* sp., *Dreshlera* sp. In November the different fungal species were as *Aspergillus niger*, *Fusarium* sp., *Aureobasidium* sp. In December, the dominating fungus species were *Aspergillus niger* sp., *Fusarium* sp

.**Open Ground:**

In the month October, fungal isolates obtained in open ground were *Aureobasidium* sp., *Curvularia* sp., *Dreshlera* sp., *Fusarium* sp. In November, the dominating fungal species were *Aureobasidium* sp., *Curvularia* sp. In December, *Dreshlera* sp., *Curvularia* sp., *Aureobasidium* sp., *Aspergillus* sp., fungal spore was obtained.

Garden:

In the month of October, fungal isolates obtained in Garden were identified as *Pythium* sp., *Curvularia* sp., *Aureobasidium* sp., *Aspergillus* sp. In November, the dominating fungal species were *Aureobasidium* sp., *Fusarium* sp., *Pythium* sp., *Dreshlera* sp. In December, the dominating fungal were *Fusarium* sp., *Aureobasidium* sp., and *Aspergillus niger*.

The study was conducted at five different sites; out of these, Library area and Office area are considered as Indoor area whereas Open Ground and Garden are considered as Outdoor area. The distribution of fungal spores in 'Indoor' and 'Outdoor' are important with respect to Aeromycology and their impact on living things. Frequency distribution of various fungus species in Indoor and Outdoor are given in Table 4 and also their percent distribution is represented graphically in figure of 13, 14 and 15 for Indoor and Outdoor and Total distribution respectively.

The study was conducted in month of October, November and December. Hence the distribution of fungal spore is representative of winter season.
In Indoor area the order of dominance is *Aureobasidium* sp. and then *Penicillium* sp., *Pythium sp, Trichoderma sp., and Fusarium sp, Curvularia* sp, and *Aspergillus* sp and last *Dreshlera* sp. In outdoor area, the order of dominance is *Aureobasidium* sp and then *Fusarium* sp. Then *Dreshlera sp, Curvularia* sp. and *A. niger* sp. then *Pythium* sp. and *Aspergillus* sp. The most frequently occurring fungal species in both Indoor and Outdoor was found to be *Aureobasidium sp. Penicillium* sp. was found only in Indoor and *A. niger* sp was observed only in Outdoor area whereas others were found to present in both Indoor and Outdoor area.

Indoor air is usually exchanged fairly and rapidly by ventilation with outside air. Due to this exchanged the microbial extent of indoor air tend to change, however, pollens and spores concentration in indoors is usually lower than outside. Indoor airs also lower than outside. Indoor air also comprises of other microbes derived from indoor sources and thrives well due to congenital environment and organic matter providing suitable substrate. A no. of species such as *Pythium* sp, *Fusarium sp, Aspergillus sp, Aureobasidium sp, Trichoderma sp, Curvularia sp, Penicillium* sp., were observed. Presence of large no. of pathogenic as well as other species can affect not only official material but also affects staff members (Tilak S.T.2009).

In parking areas, no. of fungal spore found to be present depending upon no. of environmental conditions which is suitable for growth of various species. A number of species such as *Pythium* sp. *A. Niger sp, Fusarium sp, Aureobasidium sp, Dreshlera sp and Trichoderma* sp. was demonstrated.

On ground maximum no. of fungal spores were observed. The percentage of fungal spores in air approximately 10 times that of pollen dominates. Fungal spores such as *Aureobasidium, Curvularia, Dreshlera, Fusarium, Aspergillus* which are encountered in maximum concentration, However, all these visible fungal spores in air are not allergic; some may harmless, while some may cause disease of plants and animals. The occurrence and dominance of fungal spore in air depends on variety of environmental factors such as rainfall, humidity, direction etc. According to their abundance which is determined by environmental parameters, the fungal spores have been identified as wet spora and dry spora (Tilak S.T.2009). A no. of fungal spores can be observed in near plant. In India plant disease forecasting service is at its incipient stage. The estimation and assessment of inoculums in air forms one of the major bases of devising an efficient disease forecasting system. A number of fungal spores such as *Pythium sp., Dreshlera sp., Aspergillus sp., Curvularia sp., Aureobasidium sp., Fusarium sp., Penicillium sp., A. niger sp.* were observed. Some spores were harmful to various plants, which cause serious damage to plants (Tilak S.T.2010).

Table 1: Comparative results of number of fungal isolates in three different months

Month	Sampling site	Plates	Colour of colonies on PDA plates								Total
			Blac	Gree	Red	yellow	Blue	Whit	Orang	Gray	
October	Library	1	-	-	-	-	-	3	1	-	16
		2	-	-	-	-	2	5	-	-	
		3	-	-	-	-	3	-	2	-	
	Office	1	4	-	-	-	-	5	-	8	42
		2	2	-	-	3	-	4	-	2	
		3	5	-	-	2	-	1	2	4	
	Near parking area	1	3	3	-	-	-	7	-	-	32
		2	5	4	-	-	-	2	-	-	
		3	4	3	-	-	-	-	-	-	
	Open ground	1	-	-	1	3	-	-	1	3	25
		2	-	-	3	3	-	-	-	5	
		3	-	-	1	2	-	-		-	
	Garden	1	6	-	3	2	1	3	-	4	24
		2	2	-	1	1	-	2	2	2	
		3	1	-	2	-	1	-	1	1	
November	Library	1	-	8	2	-	-	3	-	-	31
		2	-	5	2	-	-	2	-	-	
		3	-	6	3	-	-	-	-	-	
	Office	1	-	7	-	-	-	-	-	-	27
		2	-	4	-	2	-	-	-	-	
		3	-	7	-	3	4	-	-	-	
	Near parking area	1	4	-	-	-	-	-	-	-	18
		2	7	-	-	3	-	-	2	-	
		3	2	-	-	-	-	-	-	-	
	Open	1	-	-	-	4	-	-	1	-	29
		2	-	-	-	2	-	-	3	9	

	ground	3	-	-	-	3	-	-	-	7	
	Garden	1	-	-	3	2	-	5	-	-	22
		2	-	-	3	1	-	2	2	-	
		3	-	-	2	-	-	-	1	-	
December	Library	1	4	-	-	-	-	-	2	-	24
		2	5	2	-	-	2	-	-	-	
		3	1	5	-	-	1	-	2	-	
	Office	1	-	-	-	-	-	-	-	4	22
		2	-	-	-	1	-	-	2	8	
		3	-	-	-	2	-	-	-	5	
	Near parking area	1	2	-	-	-	-	-	-	-	09
		2	3	-	-	3	-	-	-	-	
		3	1	-	-	-	-	-	-	-	
	Open ground	1	5	-	2	-	-	-	-	6	26
		2	2	-	2	-	-	-	-	4	
		3	3	-	1	-	-	-	1	-	
	Garden	1	2	-	-	1	-	-	-	-	13
		2	3	-	-	2	-	-	2	-	
		3	2	-	-	-	-	-	1	-	

Table 2: Identification of fungal spores on the basis of colours.

Sr. No.	Standard Colony Colour Characteristics on PDA	Colony Colour Characteristics on PDA	Identification of Fungi
1	Blue-green, variously coloured	Green	*Penicillium sp.*
2	Colourless, white	White	*Pythium sp.*
3	Pale to bright coloured	Pale colour	*Fusarium sp.*
4	Carbon black to deep brown black	Black Colour	*Aspergillus sp.*
5	White or creamy	Cream colour	*Aureobasidium sp.*
6	Brown, gray, or black, hairy, cottony, velvety	Gray to brown colour	*Curvularia sp.*
7	Grey, brown, blackish brown, hairy, rarely velvety	Blackish brown	*Dreshlera sp.*
8	Typically shades of green of less often white, grey or brown	White with green shade	*Trichoderma sp.*

Table 3: Area and Month wise Distribution of different fungal diversity.

Sampling area	Fungi isolates		
	October	November	December
Library	*Penicillin sp.* *Pythium sp.* *Aureobasidium sp.*	*Pythium sp.* *Trichoderma sp.* *Dreshlera sp.*	*Penicillium sp.* *Aureobasidium sp.* *Trichoderma sp.* *Aspergillus sp.*
Office	*Pythium sp.* *Fusarium sp.* *Aspergillusniger.* *Aureobasidium sp.* *Curvularia sp.*	*Fusarium sp.* *Trichoderma sp.* *Penicillin sp.*	*Fusarium sp.* *Curvularia sp.* *Aureobasidium sp.*
Near Parking	*Pythium sp.* *Aspergillus sp.* *Trichoderma sp.*	*Aspergillusniger .* *Fusarium sp.* *Aureobasidium sp.*	*Aspergillusniger.* *Fusarium sp.*

Open Ground	*Aureobasidium sp.* *Curvularia sp.* *Dreshlera sp.* *Fusarium sp.*	*Aureobasidium sp.* *Fusarium sp.* *Curvularia sp.*	*Dreshlera sp.* *Curvularia sp.* *Aureobasidium sp.* *Aspergillusniger sp.*
Garden	*Pythium sp.* *Curvularia sp.* *Aureobasidium sp.* *Aspergillusniger .*	*Aureobasidium sp.* *Fusarium sp.* *Pythium sp.* *Dreshlera sp.*	*Fusarium sp.* *Aureobasidium sp.* *Aspergillusniger .*

Table 4: 'Indoor' and 'Outdoor' frequency distribution of fungal species

	Indoor		Outdoor		Total	
Fungal species	**Distribution frequency**	**% distribution frequency**	**Distribution frequency**	**% distribution frequency**	**Distribution frequency**	**% distribution frequency**
Penicillium sp.	3	14.29	0	0.00	3	05.66
Pythium sp.	3	14.29	3	09.38	6	11.32
Aureobasidium sp.	4	19.05	7	21.88	11	20.75
Trichoderma sp.	3	14.29	1	03.13	4	07.55
Dreshlera sp.	1	04.76	4	12.50	5	09.43
Fusarium sp.	3	14.29	6	18.75	9	16.98
Curvularia sp.	2	09.52	4	12.50	6	11.32
Aspergillusniger	0	0.00	4	12.50	4	07.55
Aspergillus sp.	2	9.52	3	09.38	5	09.43

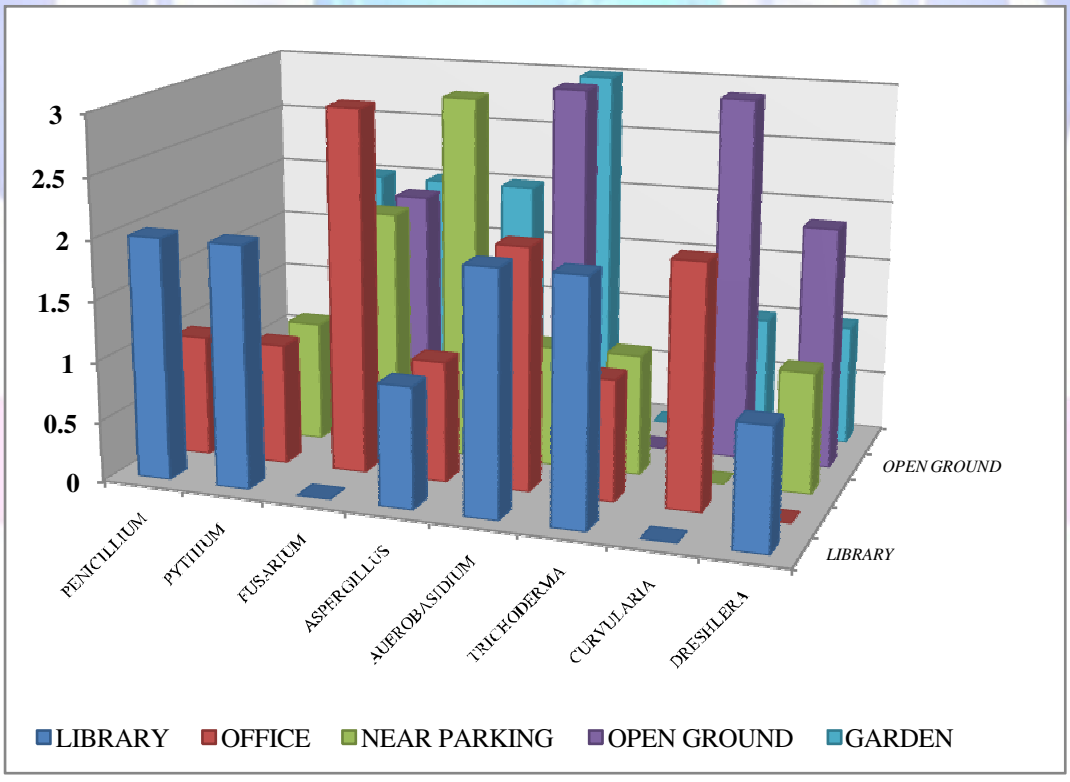

Figure. 1- Frequency distribution of various fungal species in 'Indoor and Outdoor' area.

Conclusion:

Fungal allergen exposure is associated with development and severity of asthma in sensitized individuals. The contribution of indoor fungal allergens exposure in allergic disease is still not completely clear. Method to assess fungal allergens by using immunoassays is still in their infancy. More traditional methods of exposure assessment with spore counts and quantitative cultures suggests that indoor fungal exposure indeed contributes of allergenic airway disease. The presence of fungal growth in home or offices implies a problematic measure to decrease, the infiltration of air from outdoor environment control indoor moisture problems and clean or remove contaminated material may improve health of individuals with fungal induced allergic diseases. The present study gives comprehensive overview of presence and distribution of various fungal spores in both Indoor and Outdoor areas during winter season. This study would definitely pave the way for effective management of fungal spore related problem affecting human life.

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